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Original article

Screening for *Clostridioides difficile* colonization at admission to the hospital: a multi-centre study

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ABSTRACT

Objectives: To assess the value of screening for *Clostridioides difficile* colonization (CDC) at hospital admission in an endemic setting.
Methods: A multi-centre study was conducted at four hospitals located across the Netherlands. Newly admitted patients were screened for CDC. The risk of development of *Clostridioides difficile* infection (CDI) during admission and 1-year follow-up was assessed in patients with and without colonization. *C. difficile* isolates from patients with colonization were compared with isolates from incident CDI cases using core genome multi-locus sequence typing to determine whether onwards transmission had occurred.
Results: CDC was present in 108 of 2211 admissions (4.9%), whereas colonization with a toxigenic strain (toxigenic *Clostridoides difficile* colonization [tCDC]) was present in 68 of 2211 admissions (3.1%). Among these 108 patients with colonization, diverse PCR ribotypes were found and no 'hypervirulent' PCR ribotype 027 (RT027) was detected (95% CI, 0–0.028). None of the patients with colonization developed CDI during admission (0/49; 95% CI, 0–0.073) or 1-year follow-up (0/38; 95% CI, 0–0.93). Core genome multi-locus sequence typing identified six clusters with genetically related isolates from patients with tCDC and CDI; however, in these clusters, only one possible transmission event from a patient with tCDC to a patient with CDI was identified based on epidemiological data.
Conclusion: In this endemic setting with a low prevalence of 'hypervirulent' strains, screening for CDC at admission did not detect any patients with CDC who progressed to symptomatic CDI and detected only one possible transmission event from a patient with colonization to a patient with CDI. Thus, screening for CDC at admission is not useful in this setting. **Monique J.T. Crobach, Clin Microbiol Infect 2023;29:891**

Introduction

Clostridioides difficile infection (CDI) remains an important source of health care and antibiotic-associated diarrhoea. However, not every individual develops symptomatic CDI after contact with *C. difficile* spores: patients with asymptomatic *Clostridioides difficile* colonization (CDC) outnumber symptomatic patients with CDI [1]. Patients with CDC do not exhibit symptoms but might progress to symptomatic CDI upon disturbance of their microbiota. Furthermore, they do shed *C. difficile* spores in their environment, thereby acting as a reservoir and potential source for *C. difficile* [2,3]. Although infection control measures focus currently on symptomatic cases only [4], literature has shown that isolation of patients with CDC may help in preventing nosocomial transmission

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[5]. Notably, most studies on the importance of patients with CDC are conducted in settings with high incidence rates of CDI and/or a high proportion of hypervirulent ribotypes [6,7]. The contribution of patients with CDC to the epidemiology of CDI is less well known in other settings. In this study, we investigated the value of a CDC screening programme on hospital admission in an endemic setting. Factors which determine the need for such a screening programme, including the prevalence of colonization, risk of patients with colonization to progress to CDI and chance of onwards transmission from patients with CDC to patients with CDI, were taken into account.

Methods

Study design and patients

The study was conducted at four acute-care hospitals (three university-affiliated and one general hospital) located across the Netherlands. In one of these hospitals, the Dutch reference laboratory for *C. difficile* is housed, and all hospitals participate in national sentinel CDI surveillance. In each of the four hospitals, patients were enrolled during a period of 6–8 months between January 2015 and December 2016. Adult patients admitted to predefined wards (medical and surgical) were eligible. Patients with CDI at admission or CDI diagnosed within the first 72 hours of admission were excluded. Additional exclusion criteria are listed in supplementary material (please see supplementary material). Patients could be enrolled more than once if they were readmitted during the study period. Consenting subjects had stool samples (and in one hospital, partly, rectal swabs) collected within 72 hours of admission. If patients were discharged before spending 72 hours at the hospital, stool samples could be collected at home and returned to the hospital, and no time limit was imposed on the collection of these samples. Patients with a positive *C. difficile* culture but no diagnosis of CDI were considered as having CDC. The subset of patients with CDC with a toxigenic strain in their stool cultures were considered as having toxigenic *Clostridioides difficile* colonization (tCDC). Patients with CDC were included as cases in the case-control study after obtaining written informed consent. For each case, three controls who tested negative for *C. difficile* in their stool samples obtained at admission were selected from the cohort. These controls were the three consecutive patients who submitted a study stool sample to the laboratory and agreed to participate in the case-control study.

Toxigenic *C. difficile* isolates from patients with tCDC and CDI were compared to determine whether transmission from patients with tCDC to those with CDI had occurred. The CDI cases were all hospitalized patients diagnosed with CDI during the study period and 3 months thereafter in each of the participating hospitals. The isolates of these CDI cases were collected and sent to the Dutch CDI reference laboratory as part of the national sentinel CDI surveillance [8]. All CDI cases had to comply with definitions valid in the surveillance protocol (Please see supplementary material). The test methods for the diagnosis of CDI at the four hospitals are described in the supplementary material (Please see supplementary material). Samples from recurrent (>2 but <8 weeks after the initial episode) or new (≥8 weeks after the initial episode) CDI episodes were once again included.

Microbiological analysis

Stool culture for the presence of *C. difficile* was performed on a daily basis; during weekends or holidays, the samples were stored at 4°C until the following working day. The culture methods are described in the supplementary material (Please see supplementary material). All identified isolates from (enrichment) culture were ribotyped using resolution capillary gel-based electrophoresis PCR ribotyping using the Dutch national reference laboratory library [9]. In addition, multiplex PCR was performed on cultured isolates to detect the toxin genes *tcdA*, *tcdB*, *cdtA* and *cdtB* [10]. Strains positive for *tcdA*, *tcdB* or *cdtA* or *cdtB* were defined as toxigenic strains, and all other strains were defined as non-toxigenic strains.

Data collection

Patient information was collected at baseline using a patient's questionnaire and electronic medical records. For each patient, the Charlson Comorbidity Index was calculated [11]. Follow-up using the patient's questionnaires was scheduled at 30 days and 1 year after enrolment to determine how many patients developed CDI.

Transmission analysis using core genome multi-locus sequence typing (MLST)

The methods used for re-culturing, sequencing and construction of core genome MLST are described in the supplementary material (please see supplementary material). In short, genomes were assembled as previously described [12] and annotated with Prokka [13], and alleles for core genome multi-locus sequence typing (cgMLST) were predicted using a method compatible with SeqSphere [14]. Library preparation is described in the supplementary material (please see supplementary material). Based on previous publications [15], ≤2 different alleles in cgMLST were considered to be the same strain if the time frame of sampling was <124 days and ≤3 different alleles if it was <1 year. The ward movement data of patients with CDI and colonization were investigated if their isolates were genetically related. The criteria for epidemiological linkage are described in the supplementary material (please see supplementary material).

Statistical analysis

The characteristics of patients with CDC and tCDC were compared with those of their respective controls. All analyses were performed using the STATA SE statistical software, version 15.1 (Statacorp, TX). A p value of <0.05 was considered statistically significant.

Ethical and methodological considerations

The study protocol was submitted to the institutional review ethics board, which deemed that this research is not subject to the Medical Research Involving Human Subjects Act. They had no objection to the conduction of the research or collection of the stool samples on admission under verbal informed consent. The *C. difficile* culture results were not disclosed to the patients or treating physicians. Patients selected as cases and controls provided written informed consent. Stool samples from patients with CDI were collected as part of routine care and PCR ribotyped for surveillance purposes. No additional consent was required for whole-genome sequencing of samples.

Data availability

All genomic data have been uploaded to the European Nucleotide Archive under study number PRJEB25045.

Results

Included samples and prevalence of CDC

In total, 2626 samples were screened for CDC, ranging from 500 to 1011 samples per hospital (Table 1). Of them, 415 samples were excluded from epidemiological analyses (Fig. 1). From the remaining 2211 samples, 1736 were stool samples, 467 were rectal swabs, and for eight samples, information about sampling method was lost. *C. difficile* was found in 108 samples; thus, the prevalence of CDC at admission to the hospital was 4.9% (108/2211). Toxigenic strains were found in 68 of 108 samples. The prevalence of tCDC was, therefore, 3.1% (68/2211).

Patient characteristics

In total, 194 patients were enrolled in the case-control study: 32 patients with tCDC, 17 patients with colonization by non-toxigenic strains and 145 controls (Fig. 1). The results from univariate analysis are shown in Table 2.

CDI during follow-up

None of 49 patients with colonization (95% CI, 0–0.073) or 145 control patients (95% CI, 0–0.025) developed CDI during admission or within the month after enrolment. The questionnaires at 1-year follow-up were returned by 152 (85% of alive) patients (38 patients with CDC and 114 controls). None of these patients reported to have developed CDI during follow-up (0/38 CDC; 95% CI, 0–0.093 and 0/114 controls; 95% CI, 0–0.032). A chart review of deceased patients showed than one control patient developed CDI 2 months after a negative admission screening result.

PCR ribotyping and sequence typing

Forty-four different (known) PCR ribotypes were identified among 129 patients with colonization. Colonization with the 'hypervirulent' PCR ribotype 027 (RT027) was not identified, and four patients had colonization with the 'hypervirulent' RT078 (all from different hospitals). During the study period and 3 months thereafter, 183 CDI episodes were identified, and these samples were included for comparison with tCDC isolates. RT027 was also not found among patients with CDI.

In total, 253 strains were available for the whole-genome sequencing analysis (82 isolates from patients with tCDC and 171 isolates from patients with CDI) (Fig. 1). Sequence types were assigned to all isolates (Fig. 2). ST11 (RT078, RT826 and related ribotypes) was more frequently found among patients with CDI than among patients with tCDC (19.9% vs. 4.9%, respectively; $p < 0.01$) (Fig. 2).

Table 1
Included samples and prevalence of *Clostridioides difficile* colonization and toxigenic *Clostridoides difficile* colonization per hospital

Hospital	Enrolment period	Included samples (*n*)	CDC (*n*)	tCDC (*n*)	Prevalence of CDC (%)	Prevalence of tCDC (%)	Patients enrolled in case-control study (*n*)
LUMC	Jan 2015–Jul 2015	453	19	10	4.19	2.21	44
Erasmus	Sep 2015–Apr 2016	581	36	24	6.20	4.13	50
Amphia	Oct 2015–Mar 2016	786	33	20	4.20	2.54	72
Radboud	Apr 2016–Nov 2016	391	20	14	5.12	3.58	28
Total		2211	108	68	4.88	3.08	194

Apr, April; CDC, *Clostridioides difficile* colonization; Jan, January; Jul, July; LUMC, Leiden University Medical Center; Mar, March; Nov, November; Oct, October; Sep, September; tCDC, toxigenic *Clostridioides difficile* colonization.

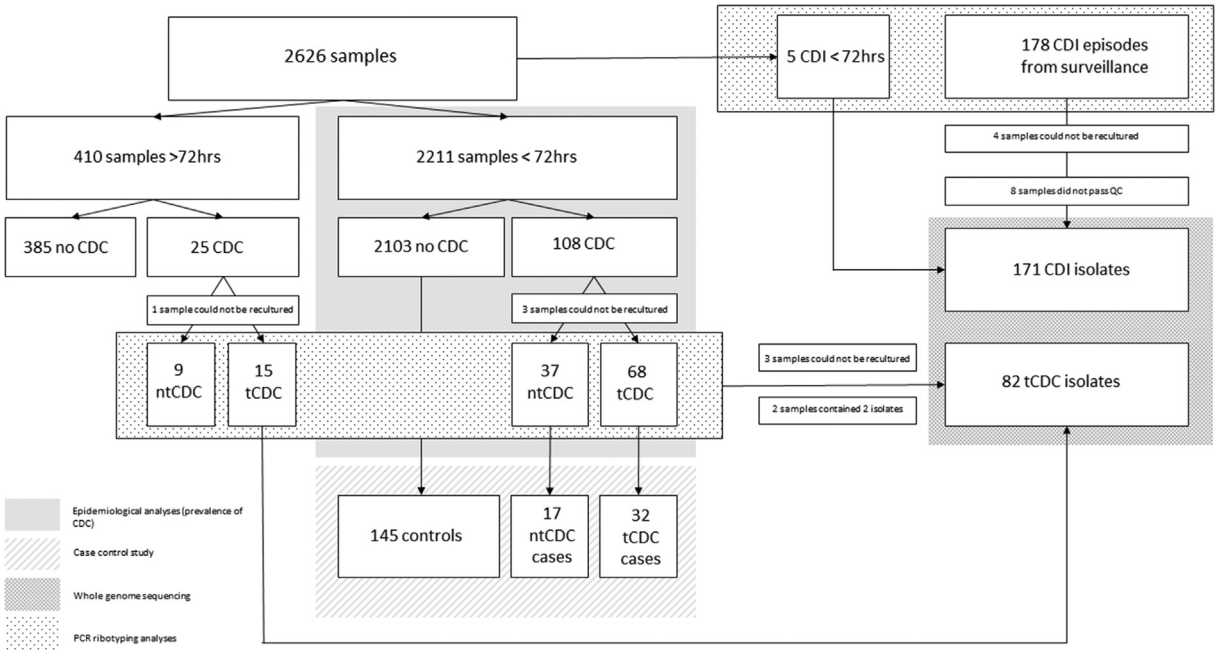

Fig. 1. Flowchart of included samples. CDC, *Clostridoides difficile* colonization; CDI, *Clostridoides difficile* infection; ntCDC, non-toxigenic *Clostridioides difficile* colonization; tCDC, toxigenic *Clostridoides difficile* colonization.

Table 2
Univariate analysis of potential risk factors for *Clostridioides difficile* colonization and toxigenic *Clostridoides difficile* colonization

Characteristics	49 patients with CDC	145 controls	Odds ratio CDC vs. control (95% CI)	32 patients with tCDC	95 controls	Odds ratio tCDC vs. control (95% CI)
Male sex	23 (46.9%)	78 (53.8%)		16 (50.0)	56 (59.0%)	
Median age (IQR)	59 (47.5–67.5)	61 (52–68)		57 (48–71)	63 (52–70)	
Born in the Netherlands	45 (91.8%)	133 (91.7%)		30 (93.8)	88 (92.6%)	
Comorbidity						
Median Charlson Comorbidity Score (IQR)	3 (2–4)	3 (1–5)		3 (2–5)	3 (1–5)	
Solid organ transplant	12 (24.5%)	15 (10.3%)	2.8 (1.2–6.5)	10 (31.3)	11 (11.6%)	3.5 (1.3–9.2)
IBD	8 (16.3%)	7 (4.8%)	3.8 (1.3–11.2)	7 (21.9)	4 (4.2%)	6.4 (1.7–23.5)
Non-metastatic solid malignancy	9 (18.4%)	25 (17.2%)	1.1 (0.5–2.5)	5 (15.6)	18 (19.0%)	0.8 (0.3–2.3)
Metastatic solid malignancy	3 (6.1%)	16 (11.0%)	0.5 (0.1–1.9)	0 (0)	10 (10.5%)	—
Chronic kidney disease	15 (30.6%)	24 (16.6%)	2.2 (1.1–4.7)	11 (34.4)	17 (17.9%)	2.4 (0.98–5.9)
DM uncomplicated	7 (14.3%)	21 (14.5%)	1.0 (0.4–2.5)	5 (15.6)	11 (11.6%)	1.4 (0.5–4.4)
DM end-organ damage	3 (6.1%)	9 (6.2%)	1.0 (0.3–3.8)	2 (6.3)	7 (7.4%)	0.8 (0.2–4.3)
Myocardial infarction	5 (10.2%)	16 (11.0%)	0.9 (0.3–2.6)	3 (9.4)	11 (11.6%)	0.8 (0.2–3.0)
Peptic ulcer disease	3 (6.1%)	11 (7.6%)	0.8 (0.2–3.0)	2 (6.3)	9 (9.5%)	0.6 (0.1–3.1)
COPD	12 (24.5%)	19 (13.1%)	2.2 (0.95–4.8)	8 (25.0)	11 (11.6%)	2.5 (0.9–7.0)
Mild liver disease	4 (8.2%)	10 (6.9%)	1.2 (0.4–4.0)	4 (12.5)	7 (7.4%)	1.8 (0.5–6.6)
Severe liver disease	2 (4.1%)	5 (3.5%)	1.2 (0.2–6.3)	1 (3.3)	4 (4.2%)	0.7 (0.1–6.8)
HIV	0 (0%)	1 (0.7%)	—	0 (0)	1 (1.1%)	—
BMT or SCT	0 (0%)	2 (1.4%)	—	0 (0)	2 (2.1%)	—
Psychiatric disorder	6 (12.2%)	17 (11.7%)	1.1 (0.4–2.8)	4 (12.5)	10 (10.5%)	1.2 (0.4–4.2)
Previous diarrhoea and CDI						
Diarrhoea in previous 3 mo	26 (53.1%)	59 (40.7%)	1.6 (0.9–3.2)	16 (50.0)	36 (37.9%)	1.6 (0.7–3.7)
Previous CDI	3 (6.1%)	1 (0.7%)	9.3 (0.9–91.9)	2 (6.3)	0 (0%)	—
Household member with previous CDI	0 (0%)	1 (0.7%)	1.0 (1.0–1.0)	0 (0)	1 (1.1%)	1.0 (1.0–1.0)
Health care contact						
Previous hospital admission within last 12 mo	36 (73.5%)	72 (49.7%)	2.8 (1.4–5.7)	24 (75.0)	47 (49.5%)	3.1 (1.3–7.5)
Working in health care system	3 (6.1%)	17 (11.7%)	0.5 (0.1–1.8)	1 (3.1)	9 (9.5%)	0.3 (0.04–2.5)
Previous medication use (last 3 mo)						
Anti-biotics	34 (69.4%)	79 (54.5%)	1.9 (0.9–3.8)	23 (71.9)	51 (53.7%)	2.2 (0.9–5.3)
PPI or antacids	36 (73.5%)	87 (60.0%)	1.8 (0.9–3.8)	25 (78.1)	56 (59.0%)	2.5 (0.98–6.3)
Anti-cancer chemotherapy	3 (6.1%)	11 (7.6%)	0.8 (0.2–3.0)	2 (6.3)	7 (7.4%)	0.8 (0.2–4.3)
Immunosuppressants	25 (51.0%)	53 (36.6%)	1.8 (0.9–3.5)	19 (59.4)	34 (35.8%)	2.6 (1.2–6.0)
Animal contact						
Pet dog	22 (44.9%)	34 (23.5%)	2.7 (1.3–5.3)	17 (53.1)	21 (22.1%)	4.0 (1.7–9.3)
Pet cat	3 (6.1%)	18 (12.4%)	0.5 (0.1–1.7)	2 (6.3)	10 (10.5%)	0.6 (0.1–2.9)
Contact with livestock	3 (6.1%)	17 (11.7%)	0.5 (0.1–1.8)	3 (9.4)	11 (11.6%)	0.8 (0.2–3.0)
Children in household attending daycare	1 (2.0%)	5 (3.5%)	0.6 (0.1–5.1)	0 (0)	4 (4.2%)	—

BMT, bone marrow transplantation; CDC, *Clostridioides difficile* colonization; CDI, *Clostridioides difficile* infection; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; IBD, inflammatory bowel disease; IQR, interquartile range; PPI, proton pump inhibitor; SCT, stem cell transplantation; tCDC, toxigenic *Clostridioides difficile* colonization.

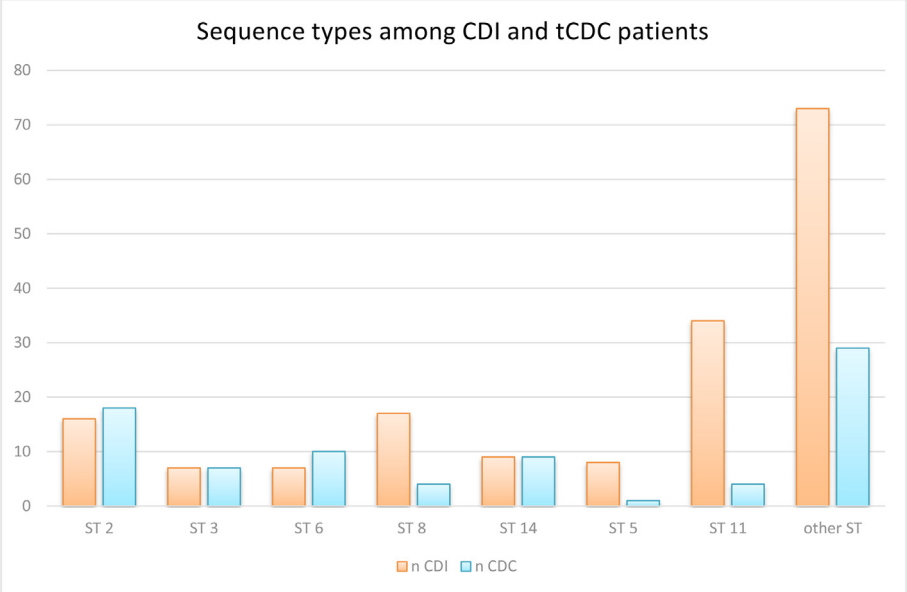

Fig. 2. Sequence types among patients with *Clostridioides difficile* infection and toxigenic *Clostridoides difficile* colonization. CDI, *Clostridioides difficile* infection; ST, sequence type; tCDC, toxigenic *Clostridoides difficile* colonization.

Core genome MLST

Given the afore-mentioned cut-offs, in total, 24 clusters could be identified (Fig. S1). Six of these clusters (C1–C6) contained isolates from patients with CDI and tCDC. The ward movement data for these clusters were investigated.

In C1 (two patients with RT020/220 from the same hospital), patients shared a ward 11 days before the first patient was found to have colonization and 37 days before the other patient was diagnosed with CDI (the direction of transmission was indeterminate). In C2 (two patients with CDI and two with CDC with RT265 from two different hospitals), a possible epidemiological link could be established between two of the patients: these patients were admitted to the same ward at the time of the first CDI positive sample and the second patient was found to have *C. difficile* colonization 42 days later at re-admission (directional transmission from the patient with CDI to the patient with CDC).

In the other four clusters (C3–C6), no epidemiological link could be found (Fig. S1).

Thirteen clusters contained isolates from CDI episodes only (Fig. S1). The largest of these clusters was earlier determined to be an outbreak of RT826 (seven samples with RT826 and one sample with RT127) at a single ward in one of the hospitals [16].

Five pairs of genetically related isolates were detected in patients with colonization (Fig. S1). Four of these pairs were identified from the same hospital >20 days apart; however, one of these pairs contained isolates from the same ward sampled only 2 days apart.

Discussion

In this multi-centre study, we screened 2211 patients at hospital admission and found that CDC was present in 4.9% of the patients at admission; colonization with toxigenic *C. difficile* strains was present in 3.1% of the patients at admission, comparable with previously reported numbers [5,17,18]. The strains identified among patients with colonization were genetically diverse, indicating various reservoirs. Sixty-three percent of patients with colonization had colonization by toxigenic strains; however, in contrast to other studies [19,20], not a single patient with colonization developed CDI in the year after study enrolment. The reason why we could not confirm a high risk of development of CDI may either be that the number of patients with colonization in our study was too low and/or that there was truly no increased risk due to a local situation such as low numbers or absence of virulent strains circulating among patients with colonization and low anti-microbial use [21].

We identified only one possible onwards transmission event from a patient with colonization: a patient with tCDC and one with CDI shared a ward before they tested positive for *C. difficile*. Our data are in contrast with published reports [6,7], which could be explained by the low-incidence setting in which our study was conducted. During the study period, the incidence of CDI ranged from 1.87 to 4.59 CDI cases per 10 000 admission days among the hospitals [8,22]. Only one outbreak due to RT826 was detected [16]. The hypervirulent RT027 was detected in neither patients with CDI nor those with CDC. Because higher transmission has been shown for certain lineages [23], the absence of these lineages may explain why transmission was infrequent in our study and why no other large clusters among patients with CDI were detected using cgMLST. Moreover, other local characteristics, such as anti-microbial pressure and infection control policies, may have played a role.

Of note, we also detected a few genetically related pairs of isolates in patients with colonization, suggesting a common source or transmission before admission, although the detection of genetically identical isolates in the same ward only 2 days apart raises the suspicion of transmission (either from patient to patient or from the hospital environment) during admission in that particular case.

Our study has numerous strengths. We captured all CDI cases because all four hospitals participate in continuous sentinel CDI surveillance. Moreover, the diagnosis of CDI was not only based on laboratory tests, but all the cases underwent a chart review by a local infection control personnel and had clinical symptoms compatible with those of CDI. We included all CDI cases which occurred in the hospitals instead of CDI cases diagnosed in study wards only because transmission may possibly extend beyond wards [24].

However, our study also has some limitations. First, we may have missed a substantial amount of *C. difficile* introduction into the hospitals because of the study's design (screening was performed on only a few specific wards per hospital) and difficulties in study execution (stool samples were only received from half of 5200 consenting subjects). During the study period, the total number of admissions in the four hospitals was 13 987, 19 424, 21 220 and 25 510, indicating that screening for colonization was not performed in the vast majority of these admissions. On the other hand, to account for *C. difficile* transmission extending beyond wards, all incident CDI cases from each hospital were included in cgMLST. Therefore, we may have underestimated the contribution of patients with colonization to overall CDI because a source could possibly not be identified if a CDI case occurred in a ward where screening for colonization was not performed.

Furthermore, patients were sampled only once during the study. Consequently, we do not know how many patients had transient colonization, were persistent carriers, or acquired colonization during admission, although this may have affected both the risk of CDI progression and *C. difficile* transmission pressure. Moreover, we only included hospital-onset CDI cases, thereby ignoring that transmission may not (directly) lead to symptomatic CDI. Patients who acquired *C. difficile* from a patient with colonization during admission but developed CDI only after discharge were not captured in our study.

Environmental swabs were not taken during our study, although patients with colonization may have contaminated the hospital environment with spores which can persist for a long time. A direct transmission link can be missing when acquisition of *C. difficile* occurs at a later moment from this contaminated hospital environment.

The criteria to determine epidemiologic linkage were quite strict and did not take into account transmission beyond wards. In our study, data about patients' movements to other hospital areas (such as the radiology department) were not available.

Another limitation includes the applied criteria to consider isolates to be the same strain because these were originally based on single-nucleotide variant analysis instead of cgMLST. Because it is not known whether the discriminatory power of both the approaches is similar, we checked all comparisons with three or less allele differences in cgMLST. In all besides one comparison, one allele was equal to one single-nucleotide polymorphism. In the last comparison, in which only one allele difference was predicted, this allele had two single-nucleotide polymorphisms. We, therefore, think that the criteria are still applicable to our study.

The implementation of screening was difficult and burdensome, whereas patients with tCDC who were detected did not have a high risk of progression to CDI themselves and were not identified as an important direct source of incident hospitalized CDI cases. However, patients with tCDC may still contribute to *C. difficile* transmission by transmitting *C. difficile* to other patients who remain asymptomatically colonized instead of (directly) progressing to CDI or by contaminating the hospital environment. The hospital environment can, however, be contaminated in many other ways, for

example, by patients with CDI whose isolation precautions are lifted after resolution of symptoms but who are still shedding spores. Therefore, we think that we should focus on decreasing CDI susceptibility (e.g. by anti-microbial stewardship programmes) and complying with general infection prevention measures to prevent the spread of *C. difficile* and other nosocomial pathogens. Sentinel surveillance to monitor the incidence rates of CDI and circulating ribotypes and the use of molecular typing in case of suspected transmission are of value for the detection of clusters and outbreaks [25]. A very typical example of this approach was the finding of the RT826 cluster, which was already detected via sentinel surveillance [16], and turned out to be the only transmission between multiple patients with CDI detected using cgMLST in this study.

Author contributions

MJTC and EJK designed the study. MJTC, EMT, CV, MCV and JH were local investigators at the four study hospitals. CH and IS performed the culturing of the isolates and DNA extraction. MDS performed DNA sequencing, genome assembly and annotation. MDS, NK and BVHH performed the bioinformatics analysis. MJTC performed the epidemiological analyses. MC and BH wrote the manuscript with the help and feedback of all other authors.

Transparency declaration

The authors declare that they have no conflicts of interest. This work was supported by the Netherlands Organisation for Health Research and Development, ZonMW (grant 50-52200-98-035) and Wellcome Sanger core funding (WT098051).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.02.022.

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